CLAIMS

1. A method for *in vivo* down-regulation of osteoprotegerin ligand (OPGL) activity in an animal, including a human being, the method comprising effecting presentation to the animal's immune system of an immunogenically effective amount of

- at least one OPGL polypeptide or subsequence thereof which has been formulated so that immunization of the animal with the OPGL polypeptide or subsequence thereof induces production of antibodies against the OPGL polypeptide, and/or
- at least one OPGL analogue wherein is introduced at least one modification in the OPGL amino acid sequence which has as a result that immunization of the animal with the analogue induces production of antibodies against the OPGL polypeptide,

whereby the animal's own OPGL is down-regulated due to binding thereof to the antibodies,

OPGL being a protein which acts as an osteoclast differentiation factor and which has an amino acid sequence as set forth in SEQ ID NO: 2 for human OPGL and in SEQ ID NOs: 4 and 6 for murine OPGL.

2. The method according to claim 1, wherein is presented an OPGL analogue with at least one modification of the OPGL amino acid sequence.

3. The method according to claim 2, wherein the modification has as a result that a substantial fraction of OPGL B-cell epitopes are preserved and that

- at least one foreign T helper lymphocyte epitope (T_H epitope) is introduced, and/or

- at least one first moiety is introduced which effects targeting of the modified molecule to an antigen presenting cell (APC) or a B-lymphocyte, and/or
- at least one second moiety is introduced which stimulates the immune system, and/or
- at least one third moiety is introduced which optimizes presentation of the modified OPGL polypeptide to the immune system.

4. The method according to claim 3, wherein the modification includes introduction as side groups, by covalent or non-covalent binding to suitable chemical groups in OPGL or a subsequence thereof, of the foreign T_H epitope and/or of the first and/or of the second and/or of the third moiety.

5. The method according to claim 3 or 4, wherein the modification includes amino acid substitution and/or deletion and/or insertion and/or addition.

6. The method according to claim 5, wherein the modification results in the provision of a fusion polypeptide.

7. The method according to claim 5 or 6, wherein introduction of the amino acid substitution and/or deletion and/or insertion and/or addition results in a substantial preservation of the overall tertiary structure of OPGL.

8. The method according to any one of claims 2-7, wherein the modification includes duplication of at least one OPGL B-cell epitope and/or introduction of a hapten.

9. The method according to any one of claims 3-8, wherein the foreign T-cell epitope is immunodominant in the animal.

10. The method according to any one of claims 3-9, wherein the foreign T-cell epitope is capable of binding to a large proportion of MHC Class II molecules.

11. The method according to claim 10, wherein the at least one foreign T-cell epitope is selected from a natural T-cell epitope and an artificial MHC-II binding peptide sequence.

12. The method according to claim 11, wherein the natural T-cell epitope is selected from a Tetanus toxoid epitope such as P2 or P30, a diphtheria toxoid epitope, an influenza virus hemagluttinin epitope, and a *P. falciparum* CS epitope.

13. The method according to any one of claims 3-12, wherein the first moiety is a specific binding partner for a B-lymphocyte specific surface antigen or for an APC specific surface antigen such as a hapten or a carbohydrate for which there is a receptor on the B-lymphocyte or the APC.

14. The method according to any one of claims 3-13, wherein the second moiety is selected from a cytokine, a hormone, and a heat-shock protein.

15. The method according to claim 6, wherein the cytokine is selected from, or is an effective part of, interferon γ (IFN-γ), Flt3L, interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 12 (IL-12), interleukin 13 (IL-13), interleukin 15 (IL-15), and granulocyte-macrophage colony stimulating factor (GM-CSF), and the heat-shock protein is selected from, or is an effective part of, HSP70, HSP90, HSC70, GRP94, and calreticulin (CRT).

16. The method according to any one of claims 3-15, wherein the third moiety is of lipid nature, such as a palmitoyl group, a myristyl group, a farnesyl group, a geranyl-geranyl group, a GPI-anchor, and an N-acyl diglyceride group.

17. The method according to any one of the preceding claims, wherein the OPGL polypeptide or subsequence thereof has been modified in any one of positions 170-192, any one of positions 198-218, any one of positions 221-246, any one of positions 256-261, or in any one of positions 285-316, the amino acid numbering conforming with that of any one of SEQ ID NOs: 4, 6, and 12, or wherein the OPGL polypeptide has been modified in any one of positions 171-193, any one of positions 199-219, any one of positions 222-247, any one of positions 257-262, or in any one of positions 286-317, the amino acid numbering conforming with that of SEQ ID NO: 2.

18. The method according to claim 17, wherein the modification comprises a substitution of at least one amino acid sequence within a position defined in claim 17 with an amino acid sequence of equal or different length which contains a foreign T_H epitope.

19. The method according to claim 18, wherein the amino acid sequence containing the foreign T_H epitope substitutes amino acids 256-261 and/or 288-302 and/or 221-241 found in SEQ ID NO: 4 or amino acids 257-262 and/or 289-303 and/or 222-243 in SEQ ID NO: 2 or in a polypeptide where a cysteine corresponding to Cys-221 has been substituted with Ser.

20. The method according to any one of the preceding claims, wherein presentation to the immune system is effected by having at least two copies of the OPGL polypeptide, the

subsequence thereof or the modified OPGL polypeptide covalently of non-covalently linked to a carrier molecule capable of effecting presentation of multiple copies of antigenic determinants.

21. The method according to any the preceding claims, wherein the OPGL polypeptide, the subsequence thereof, or the modified OPGL polypeptide has been formulated with an adjuvant which facilitates breaking of autotolerance to autoantigens.

22. The method according to any one of the preceding claims, wherein an effective amount of the OPGL polypeptide or the OPGL analogue is administered to the animal via a route selected from the parenteral route such as the intradermal, the subdermal, the intracutaneous, the subcutaneous, and the intramuscular routes; the peritoneal route; the oral route; the buccal route; the sublinqual route; the epidural route; the spinal route; the anal route; and the intracranial route.

23. The method according to claim 22, wherein the effective amount is between 0.5 µg and 2,000 µg of the OPGL polypeptide, the subsequence thereof or the analogue thereof.

24. The method according to claim 22 or 23, wherein the OPGL polypeptide or analogue is contained in a virtual lymph node (VLN) device.

25. The method according to any one of claims 1-21, wherein presentation of modified OPGL to the immune system is effected by introducing nucleic acid(s) encoding the modified OPGL into the animal's cells and thereby obtaining *in vivo* expression by the cells of the nucleic acid(s) introduced.

26. The method according to claim 25, wherein the nucleic acid(s) introduced is/are selected from naked DNA, DNA formulated with charged or uncharged lipids, DNA formulated in liposomes, DNA included in a viral vector, DNA formulated with a transfection-facilitating protein or polypeptide, DNA formulated with a targeting protein or polypeptide, DNA formulated with Calcium precipitating agents, DNA coupled to an inert carrier molecule, DNA encapsulated in chitin or chitosan, and DNA formulated with an adjuvant.

27. The method according to claim 27, wherein the nucleic acid(s) is/are contained in a VLN device.

28. The method according to any one of claims 22-27, which includes at least one administration/introduction per year, such as at least 2, at least 3, at least 4, at least 6, and at least 12 administrations/introductions.

29. A method for treating and/or preventing and/or ameliorating osteoporosis or other diseases and conditions characterized by excess bone resorption, the method comprising down-regulating OPGL activity according to the method of any one of claims 1-28 to such an extent that the rate of bone resorption is significantly decreased, such as a decrease of at least 3%, at least 7%, at least 9%, at least 11%, at least 13%, at least 15%, at least 17%, at least 20%, and at least 30%.

30. An OPGL analogue which is derived from an animal OPGL polypeptide wherein is introduced a modification which has as a result that immunization of the animal with the analogue induces production of antibodies against the OPGL polypeptide and wherein the modfication is as defined in any one of claims

17-19 while a substantial fraction of the animal OPGL B-cell epitopes is preserved.

31. An OPGL analogue according to claim 30, wherein the modification is as defined in claim 19.

32. An immunogenic composition comprising

- an immunogenically effective amount of an OPGL polypeptide autologous in an animal, said OPGL polypeptide being formulated together with an immunologically acceptable adjuvant so as to break the animal's autotolerance towards the OPGL polypeptide, the composition further comprising a pharmaceutically and immunologically acceptable carrier and/or vehicle, or
- an immunogenically effective amount of an OPGL analogue according to claim 30 or 31, the composition further comprising a pharmaceutically and immunologically acceptable carrier and/or vehicle and optionally an adjuvant.

33. A nucleic acid fragment which encodes an OPGL analogue according to claim 30 or 31.

34. A vector carrying the nucleic acid fragment according to claim 33.

35. The vector according to claim 34 which is capable of autonomous replication.

36. The vector according to claim 34 or 35 which is selected from the group consisting of a plasmid, a phage, a cosmid, a mini-chromosome, and a virus.

37. The vector according to any one of claims 34-36, comprising, in the 5'→3' direction and in operable linkage, a promoter for driving expression of the nucleic acid fragment according to claim 33, optionally a nucleic acid sequence encoding a leader peptide enabling secretion of or integration into the membrane of the polypeptide fragment, the nucleic acid fragment according to claim 33, and optionally a terminator.

38. The vector according to any one of claims 34-37 which, when introduced into a host cell, is capable or incapable of being integrated in the host cell genome.

39. The vector according to claim 37 or 38, wherein a promoter drives expression in a eukaryotic cell and/or in a prokaryotic cell.

40. A transformed cell carrying the vector of any one of claims 34-39.

41. The transformed cell according to claim 40 which is capable of replicating the nucleic acid fragment according to claim 33.

42. The transformed cell according to claim 41, which is a microorganism selected from a bacterium, a yeast, a protozoan, or a cell derived from a multicellular organism selected from a fungus, an insect cell such as an S_2 or an SF cell, a plant cell, and a mammalian cell.

43. The transformed cell according to any one of claims 40-42, which expresses the nucleic acid fragment according to claim 33.

44. The transformed cell according to claim 43, which secretes or carries on its surface, the OPGL analogue according to claim 30 or 31.

45. The method according to any one of claims 1-19, wherein presentation to the immune system is effected by administering a non-pathogenic microorganism or virus which is carrying a nucleic acid fragment which encodes and expresses the OPGL polypeptide or analogue.

46. A composition for inducing production of antibodies against OPGL, the composition comprising

- a nucleic acid fragment according to claim 33 or a vector according to any one of claims 34-39, and
- a pharmaceutically and immunologically acceptable carrier and/or vehicle and/or adjuvant.

47. A stable cell line which carries the vector according to any one of claims 34-39 and which expresses the nucleic acid fragment according to claim 33, and which optionally secretes or carries the OPGL analogue according to claim 30 or 31 on its surface.

48. A method for the preparation of the cell according to any one of claims 40-44, the method comprising transforming a host cell with the nucleic acid fragment according to claim 33 or with the vector according to any one of claims 34-39.

49. A method for the identification of a modified OPGL polypeptide which is capable of inducing antibodies against unmodified OPGL in an animal species where the unmodified OPGL polypeptide is a self-protein, the method comprising

- preparing, by means of peptide synthesis or genetic engineering techniques, a set of mutually distinct modified OPGL polypeptides wherein amino acids have been added to, inserted in, deleted from, or substituted into the amino acid sequence of an OPGL polypeptide of the animal species thereby giving rise to amino acid sequences in the set which comprise T-cell epitopes which are foreign to the animal species, or preparing a set of nucleic acid fragments encoding the set of mutually distinct modified OPGL polypeptides,
- testing members of the set of modified OPGL polypeptides or nucleic acid fragments for their ability to induce production of antibodies by the animal species against the unmodified OPGL, and
- identifying and optionally isolating the member(s) of the set of modified OPGL polypeptides which significantly induces antibody production against unmodified OPGL in the species or identifying and optionally isolating the polypeptide expression products encoded by members of the set of nucleic acid fragments which significantly induces antibody production against unmodified OPGL in the animal species.

50. A method for the preparation of an immunogenic composition comprising at least one modified OPGL polypeptide which is capable of inducing antibodies against unmodified OPGL in an animal species where the unmodified OPGL polypeptide is a self-protein, the method comprising

- preparing, by means of peptide synthesis or genetic engineering techniques, a set of mutually distinct modified OPGL polypeptides wherein amino acids have been added to, inserted in, deleted from, or substituted into the amino

acid sequence of an OPGL polypeptide of the animal species thereby giving rise to amino acid sequences in the set comprising T-cell epitopes which are foreign to the animal,

- testing members of the set for their ability to induce production of antibodies by the animal species against the unmodified OPGL, and
- admixing the member(s) of the set which significantly induces production of antibodies in the animal species which are reactive with OPGL with a pharmaceutically and immunologically acceptable carrier and/or vehicle, optionally in combination with at least one pharmaceutically and immunologically acceptable adjuvant.

51. The method according to claim 49 or 50, wherein preparation of the members of the set comprises preparation of mutually distinct nucleic acid sequences, each sequence being a nucleic acid sequence according to claim 33, insertion of the nucleic acid sequences into appropriate expression vectors, transformation of suitable host cells with the vectors, and expression of the nucleic acid sequences, optionally followed by isolation of the expression products.

52. The method according to claim 51, wherein the preparation of the nucleic acid sequences and/or the vectors is achieved by the aid of a molecular amplification technique such as PCR or by the aid of nucleic acid synthesis.

53. Use of OPGL or a subsequence thereof for the preparation of an immunogenic composition comprising an adjuvant for down-regulating OPGL activity in an animal.

54. Use of OPGL or a subsequence thereof for the preparation of an immunogenic composition comprising an adjuvant for the treatment, prophylaxis or amelioration of osteoporosis or other conditions characterized by excessive bone resorption.

55. Use of an OPGL analogue according to claim 30 or 31 for the preparation of an immunogenic composition optionally comprising an adjuvant for down-regulating OPGL activity in an animal.

56. Use of an OPGL analogue according to claim 30 or 31 for the preparation of an immunogenic composition optionally comprising an adjuvant for the treatment, prophylaxis or amelioration of osteoporosis or other conditions characterized by excessive bone resorption.